Active smoking and macrocytosis in the general population

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was excellent with a mean of 96.1% (range 95.2%-100%) of pills consumed over the 12-week treatment.

The results of this study add to the existing new evidence of the efficacy of DAAs in patients with inherited blood disorders including thalassemia. Table 1 summarizes the studies treating HCV in thalassemia patients using DAAs.[1,4–8] Importantly, the efficacy of these DAA regimens is not impacted by iron overload and is further supported by the high safety profile and lack of evidence of any significant drug–drug interaction. Early treatment of chronic HCV in patients with blood disorders should be considered in attempt to reduce liver-related morbidity and mortality.

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Active smoking and macrocytosis in the general population: Two population-based cohort studies

To the Editor:

Macrocytosis, an elevated mean corpuscular volume (MCV) of erythrocytes, is a highly prevalent phenomenon in adult individuals.[1] MCV is the measurement of the average volume of red blood cells, and macrocytosis is defined as a MCV exceeding 100 fL. Currently, in textbooks and guidelines a myriad causes are being mentioned for macrocytosis, with vitamin B_{12} and folate deficiency, alcohol use, myeloid dysplastic syndromes, and liver disease as the most prominent ones.[2] In the 70s, a number of papers have reported a positive association between smoking and MCV.[3,4] This has nowadays, however, not resulted in inclusion of cigarette smoking as an important cause of macrocytosis in textbooks and guidelines. Hence, in the current study, we aimed to investigate the association between smoking, assessed by both questionnaire and 24-hour urinary cotinine excretion, as objective measurement of nicotine exposure, with MCV in 2 large population-based cohorts.

First, we analyzed data from the Lifelines cohort study. Lifelines is a large multi-disciplinary prospective population-based cohort study which examines, in a unique 3-generation design, the health and health-related behaviors of persons living in the north of The Netherlands. For the present study, we included 131 886 of the 167 729 subjects (aged 18-93 years) of whom hematology indices, drinking and smoking behavior were available. Second, we analyzed data from the Prevention of Renal and Vascular End-Stage Disease (PREVEND) study, a prospective, population-based cohort of Dutch men and women aged 28-75 years. For current analyses, we used data from the second survey (n = 6894) and excluded missing data on smoking behavior (n = 86), resulting in 6808 participants eligible for analyses. Smoking status was categorized as never, former, and current (<6, 6-20, or >20 cigarettes/d). To exclude possible misclassification or

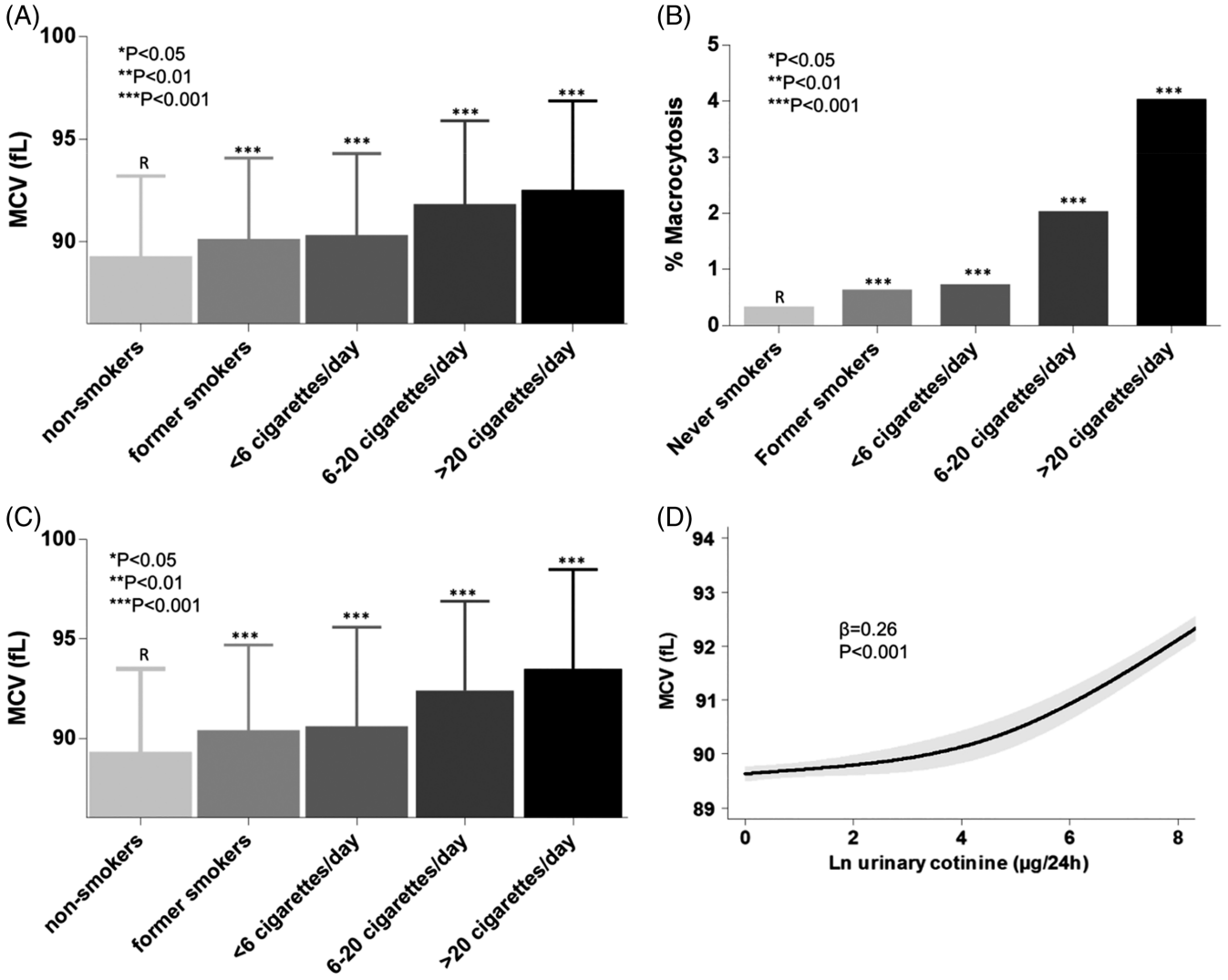

FIGURE 1 Association of smoking and 24-hour urinary cotinine excretion levels with mean corpuscular volume and macrocytosis. A, The association between smoking status and MCV in the lifelines cohort. Reported P-values are shown in respect to reference category of nonsmokers. B, The prevalence of macrocytosis for each smoking status in the lifelines cohort. Reported *P*-values are shown in respect to reference category of nonsmokers. C, The association between smoking status and MCV in the PREVEND study. Reported *P*-values are shown in respect to reference category of nonsmokers. D, The association between 24-hour urinary cotinine excretion levels and MCV by means of restricted cubic splines. Three knots have been specified at the 10th, 50th, and 90th of 24-hour urinary cotinine percentiles. The 95% CIs are indicated by the shaded areas. Twenty-four urinary cotinine levels have been natural log transformed. Abbreviations: MCV, mean corpuscular volume; PREVEND, prevention of renal and vascular end-stage disease. **P* < .05 ***P* < .01 ****P* < .001

under- or overestimation of number of cigarettes smoked per day as determined by questionnaire, 24-hour urinary cotinine levels were measured. Alcohol use was categorized as no alcohol use, 1 U of alcohol per month to 1 U/wk, >1 U/wk to 7 U of alcohol per week, >1 U/d to 3 U of alcohol per day, or > 3 U of alcohol per day. Details of the Lifelines cohort and PREVEND study regarding clinical examination, biochemical measurements, data description and statistical analyses are described in the Supporting Information. Similarly, baseline demographics and clinical characteristics of the included 131 886 community-dwelling participants and 6808 PREVEND participants are shown in Supporting Information Tables S1 and S2.

Of the 131 886 Lifelines participants (age 45 ± 13 years, 40% males), 47% were nonsmokers, 33% were former smokers and 20% were current smokers. Of the current smokers, 28% smoked <6 cigarettes per day, 55% smoked 6-20 cigarettes per day and 18% smoked > 20 cigarettes per day. Hemoglobin levels were higher in current smokers (14.3 ± 1.2 g/dL) compared with nonsmokers (14.0 ± 1.3 g/dL, *P* < .001). Similarly, MCV levels were higher in current smokers (91.4 ± 4.3 fL) compared with nonsmokers (89.2 ± 4.0 fL, *P* < .001, Figure 1A). Macrocytosis was present in 494 (1.9%) of current smokers compared with 166 (0.3%) of nonsmokers (*P* < .001, Figure 1B).

In univariable linear regression analysis, current smoking, compared with nonsmoking, was positively associated with MCV (β = 0.24, *P* < .001). In multivariable regression analysis, performed in the whole cohort, current smoking compared with nonsmoking, remained positively associated with MCV (β = 0.23, *P* < .001), independent of adjustment for age, sex, estimated glomerular filtration rate (eGFR), body mass index (BMI), and alcohol use. Multivariable regression analysis was also performed in a subgroup of participants from whom also gamma-

glutamyltransferase (GGT), alanine aminotransferase (ALAT), free thyroxine (FT4), and high-sensitivity C-reactive protein (hs-CRP) were available (n = 36 109) with the same result (β = 0.23, P < .001).

Similarly, in logistic regression, smoking was a strong determinant of macrocytosis (OR 6.25, 95% CI 5.2-7.51; P < .001 in the total cohort, OR 6.00, 95% CI 4.12-8.73; P < .001 in the subgroup of n = 36 109), independent of adjustment for potential confounders.

Hereafter, we divided current smoking into categories of cigarettes smoked per day. In multivariate analysis, all smoking categories (<6 cigarettes [β = 0.07, P < .01], 6-20 cigarettes [β = 0.22, P < .001], and >20 cigarettes [β = 0.19, P < .001]) were associated with MCV, independent of adjustment for potential confounders. The association remained the same after adjustment for GGT, ALAT, FT4, and hs-CRP (<6 cigarettes [β = 0.06, P < .001), 6-20 cigarettes [β = 0.22, P < .001], and >20 cigarettes (β = 0.21, P < .001]).

Of the 6808 subjects (age 53 $\pm$ 12 years, 50% males) in the PREVEND study, 29% were nonsmokers, 43% were former smokers, and 28% were current smokers. Of the latter, 16% smoked <6 cigarettes per day, 70% smoked 6-20 cigarettes per day, and 14% smoked >20 cigarettes per day. Hemoglobin levels were higher in current smokers (13.9 $\pm$ 1.2 g/dL) compared with nonsmokers (13.6 $\pm$ 1.3 g/dL, P < .001). Similarly, MCV levels were higher in current smokers (92.3 $\pm$ 4.7 fL) compared with nonsmokers (89.2 $\pm$ 4.3 fL, P < .001, Figure 1C). Macrocytosis was present in 73 (4%) of current smokers compared with 8 (0.4%) of nonsmokers (P < .001).

In univariable linear regression analysis, current smoking, compared with nonsmoking, was positively associated with MCV (β = 0.30, P < .001). In multivariable analysis, current smoking, compared with nonsmoking, remained positively associated with MCV (β = 0.24, P < .001), independent of adjustment for age, sex, eGFR, BMI, hs-CRP, alcohol use, GGT, ALAT, FT4, vitamin B_{12}, and folic acid. Similarly, in logistic regression, smoking was a strong determinant of macrocytosis (OR, 8.54, 95% CI 2.57-28.37; P < .001), independent of adjustment for potential confounders.

Hereafter, we divided current smoking into categories of cigarettes smoked per day. In multivariate analysis, smoking <6 cigarettes (β = 0.03, P = .06), was not associated with MCV, whereas smoking 6-20 cigarettes (β = 0.24, P < .001), and smoking >20 cigarettes per day (β = 0.13, P < .001) remained, compared with nonsmoking, associated with MCV, independent of adjustment for potential confounders.

As sensitivity analysis, we repeated in the PREVEND study the analysis with 24-hour urinary cotinine excretion levels as objective reflection of smoking. Twenty-four hour urinary cotinine excretion was strongly correlated with current smoking (β = 0.82, P < .001). Similar to the primary analysis, we identified a strong positive association between 24-hour urinary cotinine excretion and MCV (β = 0.26, P < .001, Figure 1D). The association remained independent of adjustment for potential confounders (β = 0.23, P < .001).

In this study, we have shown that smoking, assessed both by means of a self-administered questionnaire and by 24-hour urinary cotinine excretion levels, was strongly positively associated with MCV. Importantly, this association was independent of known causes of macrocytosis, including alcohol use. A few years ago, McNamee et al.[5] and O'Reilly et al.[6] reinvestigated the association between smoking as unrecognized cause of macrocytosis and showed that cigarette smoking was a significant risk factor for macrocytosis, independent of other known causes. Unfortunately, at present cigarette smoking is still not mentioned in textbooks and major guidelines, and clinicians are generally unaware of this association. The major drawback of the previously performed studies was that smoking status was assessed by means of a self-administered questionnaire, which might still be regarded as a subjective measurement of smoking status. In this study, we underline the importance of this association, and we are the first to utilize an objective measurement that is, urinary cotinine excretion levels, for the current association. The latter combined with the large patient populations can be regarded also as the major strength of this study. Due to the observational design of this study, we cannot discern potential mechanisms for the strong association between smoking and MCV. Finally, despite the extensive number of factors for which we adjust, residual confounding can still not be excluded.

In conclusion, smoking is an important determinant of MCV levels and macrocytosis, independent of prominent causes such as alcohol intake, liver disease, vitamin B_{12}, and folic acid deficiency. Smoking should be included in current guidelines regarding known causes of an elevated MCV, and the current study might draw more attention to the mechanism by which smoking causes macrocytosis independent of alcohol intake.

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CONFLICT OF INTEREST

Nothing to report.

AUTHOR CONTRIBUTIONS

All authors read and approved the final version of the manuscript. M.F.E., H.J.C.M.W., G.H. and S.J.L.B. contributed to the study design.

M.F.E. and H.J.C.M.W performed the statistical analysis. M.F.E., H.J.C.M.W., L.M.K., M.M.vd.K., P.vd.M., B.H.R.W., C.A.J.M.G., J.E.K-R., D.J.T., G.H. and S.J.L.B contributed to the interpretation of the data and analysis. M.F.E. and H.J.C.M.W. wrote the first draft and all authors edited the paper.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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Venetoclax plus decitabine induced complete remission with molecular response in acute myeloid leukemia relapsed after hematopoietic stem cell transplantation

To the Editor:

Recurrent mutations in the isocitrate dehydrogenase gene (*IDH*) have been identified in acute myeloid leukemia (AML), both in the cytosolic IDH1 enzyme and in its mitochondrial homolog, IDH2.[1] Approximately 15%-20% of AML patients have mutations in *IDH1* or *IDH2*.[2] Normal counterparts of these enzymes play their role in the citric acid cycle, catalyzing the oxidative decarboxilation of isocitrate and producing alpha-ketoglutarate; mutant IDH1 and IDH2 gain the activity to convert the alpha-ketoglutarate into 2-hydroxyglutarate, an oncometabolite that leads to epigenetic changes that promote cellular transformation through deregulation of mitochondrial function increasing BCL-2 dependence (B-cell leukemia/lymphoma-2, an antiapoptotic protein) in AML cells.[1] A subtle approach to face a malignancy with this molecular profile is to exploit the so called synthetic lethality, a strategy based on the concept of nononcogene addiction, wherein cells expressing an oncogenic mutation (ie, *IDH1/2*) exhibit dependence on subsets of nononcogenes (ie, *BCL*-2) for survival.[1] Venetoclax is a small and orally available molecule that target specifically the BH3 domain of BCL-2 (hence the name BH3 mimetic) approved for the treatment of Chronic Lymphocytic Leukemia. Several studies on the use of venetoclax as single agent in relapsed/refractory AML (r/r AML) demonstrated clinical activity with tolerable and safe profile[3]; furthermore the addition of venetoclax to decitabine or azacitidine seems to sensitize AML cells to these hypomethylating agents (HMAs), in particular for patients with IDH mutations.[4–6]

Relapsed AML after allogeneic hematopoietic stem cell transplantation (HSCT) have poor prognosis despite numerous therapies